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The Effects of Telone and Related Compounds on the Synthesis and Degradation of Carotenoids in Plants

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THE EFFECTS OF TELONE AND RELATED COMPOUNDS ON THE SYNTHESIS AND DEGRADATION OF CAROTENOIDS IN PLANTS

by

David L. Berry

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah

1971

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David L. Berry

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ABSTRACT

The Effects of Telone and Related Compounds on the Synthesis and Degradation of Carotenoids in Plants

by

David L. Berry, Master of Science

Utah State University, 1971

Major Professor: Dr. D. K. Salunkhe
Thesis Director: Dr. B. Singh
Department: Nutrition and Food Sciences

The incorporation of 3-^{14}C-β-hydroxy-β-methylglutaric acid (HMG) into carotenoids of excised etiolated maize shoots, crude cell free extracts and soluble extracts (20,000 x g) of maize was investigated. HMG was effectively incorporated into carotenoids of excised shoots, crude extracts and soluble extracts. The excised shoots, crude extracts and soluble extracts incorporated 2-^{14}C-mevalonic acid (MVA) into carotenoids as well. The results indicated the presence of HMG-CoA reductase in the plant as well as an HMG activating enzyme. The soluble extract showed a pH optimum of 7.0 for incorporation of HMG into carotenoids. Endogenous metabolites such as MVA in the soluble enzyme preparation decreased the amount of 3-^{14}C-HMG incorporated into carotenoids. The conversion of HMG to MVA may be a regulatory site in carotenoid biosynthesis in the plant.

Telone (a mixture of cis-1,3-dichloropropene, trans-1,3-dichloropropene and other halogenated hydrocarbons), 3-chloroallyl alcohol,

3-chloropropionic acid, 3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid significantly reduced the amount of 3-^{14}C-HMG incorporated into carotenoids of maize.

Activity of lipoxidase from tomato extracts was significantly inhibited by Telone, cis-1,3-dichloropropene, trans-1,3-dichloropropene, 3-chloroallyl alcohol, 3-chloropropionic acid, 3-hydroxy-propionic acid, 3-chloro-1-propanol and malonic acid.

(63 pages)

INTRODUCTION

The general use of pesticides and other agricultural chemicals is continually increasing as pressures rise for maintaining food, feed and fiber productivity on reduced acreages. Agricultural chemical sales amounted to $1.7 billion in 1968 (Neumeyer, Gibbons and Trask, 1969). The fumigants amounted to about 8% of the total sales and over $19 million of the total was exported. Many of the fumigants that were developed and marketed prior to 1960 are still in use and little is known about their mechanisms of action and their effects on non-target organisms.

Recent evidence has shown that the small molecular weight, volatile, halogenated hydrocarbons used commercially as fumigants in the soil give rise to beneficial changes in the plant grown on treated soil. Of particular interest is the increased carotenoid level in green and yellow vegetables (corn, carrots and peas) produced on soils treated with Telone (Wu et al., 1970).

Telone (a mixture of cis-1,3-dichloropropene, trans-1,3-dichloropropene and other chlorinated hydrocarbons) has been used commercially as a soil fumigant to control root knot nematodes, lesion nematodes, cyst formers and golden nematodes and other species. Telone alters the soil microflora population and inhibits the growth and population of the nitrification bacteria (Altman and Lawlor, 1966). In turn, plants able to utilize ammonium nitrogen show increased vigor and growth.

The mechanism of increased carotenoid level cannot be explained by the above factors. Telone may somehow alter or affect the plant's metabolism so that it produces more carotenoids. Three distinct possibilities may account for the increased carotenoid levels. First, Telone or one of its soil metabolites, causes an increase in the rate of biosynthesis of carotenoids in the plant. Secondly, Telone or one of its soil metabolites, decreases the rate of degradation of carotenoids in the plant. Finally, the plant may absorb Telone or one of its soil metabolites and further metabolize the unknown compound to cause a direct change in the plant's secondary metabolism.

The aim of this study was twofold: first, to study the effects of Telone and related compounds on the biosynthesis rate of carotenoid formation in the maize plant; second, to investigate the effects of Telone and related compounds on the degradation of carotenoids in the plant. The use of β-hydroxy-β-methylglutaric acid as a precursor to carotenoids in the plant and the existence of β-hydroxy-β-methylglutaryl coenzyme A reductase will be examined.

REVIEW OF LITERATURE

The role of β-hydroxy-β-methylglutaric acid (HMG) as an intermediate in the synthesis of isoprene units in animals and the protista such as yeast and bacteria has clearly been established by Brodie and Porter (1960), Brodie, Wasson and Porter (1963), Brodie, Wasson and Porter (1964), Burch, Rudney and Irias (1964), Bucher, Overath and Lynen (1960), Durr and Rudney (1960), Ferguson, Durr and Rudney (1958), Kawachi and Rudney (1970), Kirtley and Rudney (1967), Knappe, Ringelmann and Lynen (1959), Knauss, Porter and Wasson (1959), Linn (1967a), Linn (1967b), Lynen (1967), Rudney et al. (1966), Stewart and Rudney (1966a), Stewart and Rudney (1966b) and White and Rudney (1970). The enzyme β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (E.C.1.1.1. 34) has been isolated from liver homogenates and yeast extracts. This enzyme is responsible for the reduction of β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid (MVA), the precursor to the basic isoprenoid unit Δ^3-isopentenyl pyrophosphate.

A direct regulatory role in steroid biosynthesis has been established for HMG-CoA reductase by Linn (1967a), Linn (1967b) and Siperstein and Fagan (1966) in rat liver. The enzyme rate is controlled by the level of sterols in the homogenate (a form of feed back inhibition) and the enzyme in rats shows a cyclic pattern. Kirtley and Rudney (1967) have isolated HMG-CoA reductase from yeast and were able to show that it had followed the kinetics of the classical

"ping-pong" mechanism or allosteric behavior. Such evidence would indicate that in yeast and mammals, HMG-CoA reductase is a regulatory enzyme.

In higher plants, however, very little is known about the role of HMG as a precursor of isoprenoid compounds. Archer et al. (1963), Chesterton and Kekwick (1968), Goodwin (1969), and Porter and Anderson (1967) have pointed out conflicting views for the existence of HMG-CoA reductase and the role of HMG in the plant. Recently, Hepper and Audley (1969) were able to show that HMG-CoA was converted into MVA by a soluble fraction from latex in rubber plants. They were able to show that the enzyme HMG-CoA reductase was active in the plant and activity varied with endogenous MVA levels and season. By contrast, Potty (1969) was unable to show any evidence for the conversion of HMG or HMG-CoA into MVA in mature orange vesicles.

Unlike the synthesis of isoprenoids, the degradation of carotenoids has been well established. The enzyme lipoxidase (lipoxygenase) which catalyzes the conversion of the cis, cis-1, 4-pentadiene system to its corresponding hydroperoxide of the cis, trans configuration has been clearly defined by Goodwin (1964), Grossman et al. (1969), Kies et al.(1969), Tappel (1962) and Surrey (1964). Lipoxidase has been assigned the major role of degradation of carotenoids in the plants by Goodwin (1964). Only a slight amount of carotenoids are degraded by light in the plant as suggested by Seeley and Meyer (1971).

The effect of pesticides on the biosynthesis and degradation of carotenoids in the plant has not been heretofore examined. In fact, the agricultural chemicals marketed before 1960 and that are still in use have had little research on their effects on non-target organisms. Telone (a product of Dow Chemical Company and a mixture of cis- and trans-1,3-dichloropropene and other halogenated hydrocarbons) is commonly used as a soil fumigant and has been used since its introduction in 1956 by Fletcher (1956). Work by Salunkhe et al. (1971) and Wu et al. (1970) have indicated that the plants grown on soils treated with Telone and Nemagon (1,2-dibromo-3-chloropropane) have higher carotenoid levels than untreated plants.

Several researchers have studied the physical factors that affect Telone and other organochloride (OC) compounds in the soil. Castro (1966) has shown that once Telone is exposed to the soil, it is quickly hydrolyzed to its corresponding 3-chloroallyl alcohol and remains in that stable form as a biocide. Jurinak (1957) and Jurinak, Brown and Martin (1960) have shown that 1,2-dibromo-3-chloropropane and ethylene dibromide are bound to the surface of clay and organic soil particles. Lichtenstein et al. (1970) have shown that loam soils treated with Aldrin and Heptachlor retain residues for as long as ten years. Leistra (1970) studying the distribution of 1,3-dichloropropene in soils has noted that the trans isomer is more easily adsorbed to soil. In addition, peat soil adsorbs the highest level of 1,3-dichloropropene. Wheeler (1970), Williams (1968), and Lichtenstein and Schulz (1959) have found that the nature of the chemical, the soil type, the micro-environment of the soil, the

temperature, the relative humidity and the nature of the plant cover are important factors in controlling the amount of pesticide level in a given soil.

Almost from the time the halogenated hydrocarbon fumigants became available for use, changes in plant growth on treated soils were noted. Tams and Clark (1943) and Tams (1945) working with pineapple noted that plants had more vegetative growth and higher leaf nitrate levels. Theigs (1955), Martin and Pratt (1958), and McCants, Skogley and Woltz (1959) noted that fumigated soils had higher ammonium nitrogen levels and that they could be toxic to plants assimilating only nitrate nitrogen. Wolcott et al. (1960) indicated that Telone treated soils had higher ammonium nitrogen and these levels of ammonium nitrogen were toxic to celery plants. Altman and Tsue (1966), English et al. (1961) and English and Devay (1964) stated that sugar beets and stone fruit trees had more vigor when grown on fumigated soil. Cole et al. (1968) and MacKenzie et al. (1968) noted similar evidence for plant vigor and examined micronutrient levels in the soil. Not only did they find changes in levels of Mg, Fe, Cu, and Zn in the soil but also in the soil microbial population.

Altman and Lawlor (1966), Moje, Martin and Baines (1957), Moje (1959), Wensley (1953), Whitehead, Tite and Fraser (1970a), and Whitehead, Tite and Fraser (1970b) have reported changes in the type of soil microbes present in the fumigated soils. Of particular interest were the increases in some of the fungal populations and the decreases in the nitrifiers of the soil. Caseley and Broadbent (1968) stated that not only were there changes in the

microbial populations but also changes in soil respiration with the fumigant Lanstran (1-chloro-2-nitropropane). The most interesting work associated with the microbial changes in the soil are those of enzymes found in certain microorganisms that dehalogenate and modify many of the fumigants. Belser and Castro (1971), Castro and Bartnicki (1965), Castro and Bartnicki (1968), Castro and Belser (1968), and Bartnicki and Castro (1969) have found a series of enzymes in Pseudomonas sp. which are capable of dehalogenation. Goldman, Milue and Keister (1968) and Kearney, Kaufman and Ball (1964) have described a similar reaction and have postulated possible mechanisms for the reaction.

Lichtenstein (1960), Lichtenstein and Schulz (1960), Lichtenstein, Millington and Cowley (1962), Lichtenstein and Schulz (1965), Lichtenstein and Myrdal (1965) and Lichtenstein et al. (1967) have reported changes in levels of some of the organochlorine insecticides in the plant. They have described adsorption of these compounds by root crops and their subsequent translocation throughout the plant. Harris and Sans (1967) found similar results with sugar beets and other root crops. Saha and McDonald (1967) and Saha, Craig and Junzen (1968) described adsorption of organochlorine compounds in wheat and legumes. Cotner et al. (1968) reported certain organochlorine chemicals are adsorbed and translocated to the xylem, trachieds and adjacent mechanical tissue. Maier-Bodie (1967) reported high levels of Dieldrin in carrots from Germany. Gabelman (1970), working on a genetic problem, has postulated that root crops such as carrots may be used to decontaminate soils having high levels of organochlorine compounds.

As Casida and Lykken (1969) pointed out, very little was known about the metabolism of the compounds once they have entered the plant. The organochlorine compounds are particularly lacking in plant metabolism studies. Lichtenstein and Corbett (1969) and Oloffs and Lichtenstein (1969) have described the epoxidation of Aldrin to Dieldrin in certain root crops and have isolated a subcellular component from peas that carries out the epoxidation.

Changes in the secondary plant products, such as carotenoids, have been noted recently by Wu et al. (1970) and Salunkhe et al.(1971). In addition, changes in the size and shape of chromoplasts (storage body in the cell for carotenoids) have been noted by Wu and Salunkhe (1971).

EXPERIMENTAL

Solvents

Petroleum ether (b.p. 40-60 C), ethyl ether, and acetone were J. T. Baker Reagent Grade. (Petroleum ether and ethyl ether were distilled over reduced iron and stored over anhydrous Na_2SO_4.) Toluene, methanol and chloroform were J. T. Baker Spectrophotometric Grade.

Chromatographic adsorbents

Magnesium oxide (Chromatographic Grade) was obtained from J. T. Baker, Phillipsburg, New Jersey, and Hyflo Super-Cel (Chromatographic Grade) was obtained from Johns-Manville, Lompoc, California.

Radiosotopes

DL-2-^{14}C-mevalonic acid (MVA) (dibenzylethylenediamine salt, 5.90 mC/mM) and 3-^{14}C-β-hydroxy-β-methylglutaric acid (HMG) (3.2 mC/mM) were obtained from New England Nuclear, Boston, Massachusetts, 3-^{14}C-HMG CoA was prepared by the method of Hilz et al. (1958).

Chemicals

Coenzyme A (CoA) (free acid) was obtained from Calbiochem, Los Angeles, California. Glutathione, Linoleic acid (Grade III), $NADH_2$, $NADPH_2$, NAD^+ and $NADP^+$ (all Grade III) were obtained from Sigma Chemical Co., St. Louis, Missouri, α and β-carotene standards were obtained from

Eastman Chemical, Rochester, New York. Malonic acid, 3-chloroallyl alcohol (3-Cl-allyl alcohol), 3-chloropropionic acid (3-Cl-propionic), 3-hydroxypropionic acid (3-OH-propionic) and 3-chloro-1-propanol (3-Cl-1-propanol) were obtained from K & K Laboratories, Inc., Plainview, New York. Cysteine, polyvinyl pyrrolidone (K-30) and 2-mercaptoethanol were obtained from Nutritional Biochemicals, Cleveland, Ohio. Triton X-100, 2,5-diphenyloxazole (PPO) and ρ-bis-{2-(4-methyl-5-phenyloxazolyl)} benzene (dimethyl-POPOP) were obtained from Packard Instrument Company, Inc., Downers Grove, Illinois. Telone, cis-1,3-dichloropropene (cis-1,3-dcp) and trans-1,3-dichloropropene (trans-1,3-dcp) were supplied by Dow Chemical Company, Midland, Michigan.

Preparation of the tissues

Corn seedlings (Zea mays L. cultivar Iochief) were grown in vermiculite in dark. The etiolated seedlings were harvested when 10 days old. For excised shoot studies, the seedlings were excised above the first node and the shoot was suspended in a flask containing Hoagland's solution (Arnon and Hoagland, 1940) to which a measured quantity of radioisotope was added. Crude extracts were prepared by homogenizing 100 g of excised shoot tissue in a Sorvall Omni-Mixer with 400 ml of 0.05 M phosphate buffer, pH 7.0, at 4 C. Soluble extracts were prepared by filtering the crude extract through four layers of cheese cloth and centrifugation of the resulting liquid at 20,000 x g for 15 min in a Sorvall SS 1 refrigerated centrifuge. The soluble extracts were dialyzed

against a 0.001 M phosphate buffer, pH 7.0, at 0 to 4 C for 12 hr in 2" dialysis tubing. Buffer changes were every 3 hr.

Reaction mixtures

In the excised tissue experiments, 2 to 5 g of excised shoots were incubated in 19 ml Hoagland's solution containing 1.16×10^5 disintegrations per minute (dpm) 3-^{14}C-HMG or 2.4×10^5 dpm 2-^{14}C-MVA. (For Telone and related compound investigations, measured quantities were added to the afore mentioned mixture.)

Fifteen milliliters of crude extract were incubated in a 50 ml Erlenmeyer flask to which 1.16×10^5 dpm 3-^{14}C-HMG or 2.4×10^5 dpm 2-^{14}C-MVA was added.

Unless otherwise stated, the assay system for the soluble maize extract consisted of 1.16×10^5 dpm 3-^{14}C-HMG, 5 μmole glutathione, 1 μmole Co A (free acid), 4 μmole ATP and 5 μmole $NADP^+$ and 9.5 ml soluble maize extract in a total volume of 10 ml. In addition, the dialyzed extract contained 5 μmole Mn^{+2}, 5 μmole Mg^{+2}, 5 μmole $NADPH_2$, 5 μmole NAD^+ and 5 μmole $NADH_2$ in a total volume of 10 ml.

In every case, the incubation was at 25 C under continuous illumination and shaking for 12 to 72 h.

Extraction of lipids

Tissues were homogenized with 15 ml cold acetone in an Omni-Mixer. They were extracted 3-4 times, each with 15 ml of cold acetone. The combined

homogenates were extracted exhaustively 3-4 times with ethyl ether (50 ml portions) and washed with water so that no detectable acetone remained in the ether extract. The combined ether extracts were saponified by method of Goodwin (1964), except that saponification was carried out at 25 C overnight and without heating. The saponified extracts were washed with water until neutral and then dried using anhydrous Na_2SO_4 overnight at -5 C. The water free extract was evaporated to dryness in vacuo, diluted with a minimum volume of petroleum ether, and quantitatively transferred to 25 ml Erlenmeyer flasks and evaporated to dryness under N_2. The crude extract and the soluble maize extract were extracted for carotenoids in a similar fashion.

Separation of carotenoids and non-saponifiable ether-soluble material

The samples were dissolved in a minimum volume of solvent and spotted on thin layer chromatography plates, 20 x 20 cm. The adsorbent was Magnesium Oxide:Hyflo Super-Cel (1:2;w/w) spread 0.5 mm thick on glass plates. The solvent system was benzene:petroleum ether (60:40;v/v). Separation was carried out in closed chambers in the dark. The solvent and adsorbent system allowed separation of the various carotenoids with respect to the number of C_5 units, α or β-ionone rings and ring substitutions. Separation with very little tailing allowed fine resolution of the products. Pigments were scraped from the plates and eluted off the adsorbent with ethyl ether. The pigments were identified spectrophotometrically in a Beckman DB-G recording spectrophotometer. Aliquots of the carotenoids were prepared for counting in a liquid scintillation counter.

Lipoxidase assay

Fifty grams of mature, red, ripe tomatos (Lycopersicon esculentum L. Experimental cultivar VF-7) were homogenized in 200 ml of 0.067 M Tris-HCl buffer, pH 7.5, at 4 C. The homogenate was filtered through four layers of cheese cloth and centrifuged at 12,000 x g for 30 min in an International B 20 refrigerated centrifuge. The resulting supernatant was used as a source of lipoxidase activity.

The assay mixture, unless otherwise stated, consisted of 7.5 μmoles linoleic acid (in the form of ammonium linoleate) 67 μmoles of Tris-HCl buffer, pH 7.5, saturated with oxygen, 1.0 ml of the soluble tomato extract and distilled water to a total volume of 3.5 ml. (Telone and related compounds were added to the assay mixture when activity studies were made.) The reaction was followed spectrophotometrically at 232.5 nm in a Perkin Elmer Model 124 Spectrophotometer equipped with a cell programmer and reaction rate recorded for 10 min. The assay method was that of Surrey (1964).

Radioassay

Samples were placed in counting vials, decolorized with 0.5 ml of 5% sodium hypochlorite solution and then 15 ml of scintillation mixture was added. The scintillation mixture contained 5.0 g PPO, 0.3 g dimethyl-POPOP, 333 ml Trition X-100 and toluene to 1 liter. Scintillation counting was carried out on a Packard Model 527 Tri-Carb Spectrophotometer. Corrections were made for hypochlorite quenching using a standard quench curve.

Radioautography

Samples were spotted in TLC plates, developed, dried, covered with Saran wrap and placed in contact with X-ray film for 10-14 days.

Statistical analysis

Analysis of variance was completed and the means were compared according to Tukey's ω-procedures (Sokal and Rohlf, 1969).

Protein determination

Soluble protein was determined by method of Lowry et al. (1951).

RESULTS

Incorporation of 3-^{14}C-HMG into carotenoids by the excised etiolated maize shoots

The radioactivity from 2-^{14}C-MVA, as well as from 3-^{14}C-HMG, appeared in carotenoids of excised etiolated maize shoots (Table 2). Up to 60% of the label (based on the L isomer) from MVA appeared in the non-saponifiable fraction during 36 hr of incubation at 30 C. Incorporation of the label from 3-^{14}C-HMG into the non-saponifiable fraction approached 10% or one-sixth that of MVA (Tables 2, 3). Although other isoprenoids including sterols, squalene, geranyl geraniol and terpenoids would be expected to contain label from both HMG and MVA, analysis was based only on the carotenes.

Incorporation of 3-^{14}C-HMG into carotenoids by cell-free extracts

Crude extracts

Crude extracts of etiolated, excised maize seedlings incubated with 0.05 M phosphate buffer, glutathione, continuous illumination and shaking incorporated HMG into carotenoids. The total amount of carotenoid formed was less and the type of carotenoids synthesized differed from that of the excised shoots (Figure 1). The crude maize extract incorporated about 20% of the MVA label into the carotenoid fraction (based on L isomer). The major carotene formed by the crude extract appeared to be β-carotene in

the case of MVA incubation and perhaps phytoene in the case of HMG incubation (Figure 1).

Soluble extract

A soluble extract of etiolated maize (20,000 x g) in a 0.05M phosphate buffer, glutathione, NADP, ATP and CoA (free acid) incorporated HMG into the whole carotenoid fraction. The soluble system behaved similar to the crude extract in that less carotenoid was formed in the non-saponifiable fraction and β-carotene was the predominant carotene present.

The soluble system had a pH optimum for incorporation of HMG into carotenoids of 7.0 (Figure 2). At pH values higher than 7.5 or lower than 6.0, the incorporation of HMG into carotenoids decreased rapidly.

Ammonium sulfate fractionation

Ammonium sulfate fractionation of the soluble maize extract showed that most of the HMG incorporating activity was contained in the 36 to 65% saturation fraction. No further attempts were made to purify the system beyond ammonium sulfate fractionation.

Effect of cofactors and other agents in the soluble maize fraction

The effect of ATP, NADP, $NADPH_2$, NAD and $NADH_2$ on the incorporation of HMG into carotenoids in the soluble system was difficult to determine because of the existing pool sizes of these substances. However, the system did show a definite dependence on the presence of reduced glutathione (GSH)

(Table 5). The use of 10 ml of 10% protamine sulfate per 80 ml soluble maize extract had no effect on the activity of the system; however, a decrease in the soluble protein level did occur. The use of insoluble polyvinyl pyrrolidone (0.5 gm/g fresh weight maize tissue) or Na_2SO_3 (2 ml of a 0.5% solution per 100 ml) was necessary to prevent inhibition of the system by polyphenols present in the maize extract.

Dialysis of the soluble extract

Dialysis of the soluble maize extract over a period of 12 hours against a 0.001 M phosphate buffer at 4 C resulted in complete inability to incorporate HMG into carotenoids. Addition of various cofactors to the dialyzed fraction could not restore activity to the extract.

Effect of MVA on incorporation of HMG into carotenoids

The excised, etiolated maize tissue and the soluble extract exhibited a lower rate of incorporation of 3-^{14}C-HMG and 3-^{14}C-HMG CoA into carotenoids in the presence of endogenous MVA. The excised tissue in the presence of 10 μmoles of MVA showed a decreased incorporation of 3-^{14}C-HMG as compared with tissue incubated with HMG alone. The soluble extract displayed similar behavior in the presence of 5 μmoles MVA (Table 4).

Effect of Telone and related compounds on incorporation of 3-^{14}C-HMG into carotenoids

Excised, etiolated maize shoots incubated in Hoagland's solution containing a known amount of 3-^{14}C-HMG were used to study the effects of Telone and related compounds on the synthesis of carotenoids. Low concentrations of Telone, 3-chloroallyl alcohol, 3-chloropropionic acid, 3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid were administered to incubating shoots and after 36 hours, the shoots were homogenized and the carotenoids extracted (Table 6, Figure 4). All concentrations of Telone applied to the incubation mixture were significantly inhibitory to carotenoid biosynthesis. Of the three concentrations of 3-chloroallyl alcohol applied to the incubation mixture, two significantly inhibited carotenoid biosynthesis. Only the high treatment levels of 3-chloropropionic acid significantly decreased the incorporation of HMG into carotenoids. Application of 3-chloro-1-propanol and 3-hydroxypropionic acid at all levels significantly decreased HMG uptake into carotenoids of the shoots.

Effect of Telone and related compounds on the incorporation of 2-^{14}C-MVA into carotenoids

Excised, etiolated maize shoots incubated in Hoagland's solution containing a known amount of 2-^{14}C-MVA were used to study the effects of Telone and related compounds on the uptake of MVA into carotenoids. Low concentrations of Telone, 3-chloroallyl alcohol and malonic acid were administered to the incubating shoots. After 36 hours, the shoots were homogenized and the

carotenoids extracted (Table 7, Figure 5). The three compounds produced significantly lower quantities of carotenoids compared to untreated control shoots.

Effect of Telone and related compounds on the activity of lipoxidase

A soluble enzyme preparation from tomato was used as a source of lipoxidase activity. Concentrations of Telone, cis-1,3-dichloropropene, trans-1,3-dichloropropene, 3-chloroallyl alochol, 3-chloropropionic acid, 3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid were added to the reaction mixtures and the effect on in vitro enzyme activity were noted (Table 8, Figure 6). Telone and cis-1,3-dichloropropene produced the largest decrease in lipoxidase activity of the compounds tested. The concentrations of 3-chloropropionic acid and trans-1,3-dichloropropene produced erratic results with respect to inhibition patterns of lipoxidase. The 3-chloroallyl alcohol was a very potent inhibitor of lipoxidase activity. The concentrations of 3-chloro-1-propanol and malonic acid were found to be inhibitory to lipoxidase activity as well.

DISCUSSION

The incorporation of 3-^{14}C-HMG into carotenoids upon incubation with excised etiolated maize shoots, crude extracts of maize shoots and soluble extracts of maize shoots suggests that HMG is a precursor of carotenoids in plants. Cooper and Benedicts (1967), Hill, Shah and Rogers (1969) and Shah and Rogers (1969) have found that label from acetate, acetyl-CoA and acetoacetyl-CoA was incorporated into carotenoids at a rate of 1 to 4% of the total label administered. The results presented in this paper indicate that the label from HMG is incorporated into carotenoids at a rate of 7 to 11% of the total applied label. HMG was incorporated into carotenoids at about one-sixth of the efficiency of MVA.

The soluble extract system incorporated HMG into the non-saponifiable ether soluble fraction at a lower rate than the crude extract. Centrifugation at 20,000 x g would eliminate most of the intact chloroplasts which Shah and Rogers (1969) have shown to be the site of carotenoid synthesis in the plant. The HMG incorporation activity apparently was imparted to the soluble extract during homogenization of the excised shoot. The ruptured chloroplast was probably the source of enzyme activity.

The soluble extract incorporated 3-^{14}C-HMG as well as 12-^{14}C-MVA into carotenoids. The CoA ester form of HMG was incorporated at about the same efficiency as the free acid form of HMG. These results would indicate that both forms are treated equally by the soluble system and are

apparent intermediates in the pathway. The rate of incorporation of HMG, however, was lower than that of MVA. Moreover, the rate of incorporation of 3-^{14}C-HMG into carotenoids decreased in the presence of unlabeled additional MVA by excised shoots, crude maize extracts and soluble maize extracts. These results indicate that MVA is preferntially utilized over HMG as a source of isoprene precursor in carotenoid biosynthesis. Additionally, MVA level may be controlling the conversion of HMG into MVA in the plant.

The incorporation of HMG into carotenoids is contrary to previous studies by Hepper and Audley (1969) and Potty (1969) which have shown that it is the CoA form of HMG that is incorporated into isoprenoids and not the free acid form. In mammals and yeast, Brodie and Porter (1960), Brodie, Wasson and Porter (1963), and Rudney et al. (1966) have shown that HMG CoA or a bound form of HMG may be the intermediate that is reduced to MVA. In fungi, Yokoyama, Nakayama and Chichester (1962) have shown that the free acid is incorporated into β-carotene by a cell free extract requiring CoA. The soluble system in maize also showed a higher activity in the presence of CoA (free acid) and $NADP^+$. The CoA effect may be due to the increase in the pool size, the formation of HMG-CoA via a thiol transferase reaction as postulated by Rudney et al. (1966) or a direct activation of HMG by a thiolase.

The possibility that HMG is degraded down to acetyl-CoA and then taken back up to MVA is eliminated when one observes the amount of label incorporated into the carotenoids. The incorporation of the label into

β-carotene in the shoots, crude extracts and soluble extracts would be lower by a factor of 5-10 fold if HMG followed the pathway : HMG - - → acetyl-CoA ----→ MVA ---→ carotenoids. Since from 7-11% of the label of 3-^{14}C-HMG is found in the carotenoids, HMG probably goes directly to MVA and into isoprenoids rather than acetyl-CoA and be shunted to other compounds as well as isoprenoids.

The soluble system showed a marked dependence on reduced glutathione or cysteine. Activity of the extract decreased rapidly without GSH or cysteine even at 0 C. The use of either GSH, 2-mercaptoethanol or cysteine during extraction and centrifugation was essential to maintain activity of the enzyme.

The presence of polyphenols in the soluble extract inhibited the incorporation of HMG into carotenoids. To control the inhibition of the polyphenols, either insoluble polyvinyl pyrrolidone was added to adsorb the polyphenols or Na_2SO_3 was added to the extract to inhibit polyphenol oxidase. The Na_2SO_3 was not inhibitory to the HMG incorporating system and effectively controlled browning in the reaction mixture.

Dialysis of the soluble extract either for 12 hours or even for 4 hours resulted in complete loss of enzyme activity. At first thought, the loss of one or more cofactors was suspected. However, addition of all known cofactors to the enzyme system could not restore activity. The enzyme system which converts HMG to MVA in the plant appears to be labile and easily inactivated during dialysis.

It is entirely possible that in plants as in animals, HMG or HMG-CoA is an intermediate in the synthesis of isoprenes. This implies the existance of an HMG activating enzyme in the etiolated maize shoots, in addition to HMG-CoA reductase. Lynen (1967) has shown that in rubber latex, the conversion of HMG-CoA to MVA is a rate limiting step in isoprenoid biosynthesis. Since HMG-CoA reductase may be a regulatory site in carotenoid biosynthesis, the significance of these results may be of interest. The actual form of the intermediate is presently unknown; however, the results would indicate that HMG is effectively incorporated into isoprenes as a unit.

Since the conversion of HMG into MVA is potentially a site of regulation in carotenoid biosynthesis, it is of interest to determine the effects of other applied chemicals on that site. Particularly, when an increase in carotenoid level occurs in plants grown on Telone treated soils. Additionally, the effect of the chemical may be on the degradation of carotenoids, so a study to ascertain the effects of Telone on that site was included as well.

The potential soil metabolites of Telone are listed in Figure 3. Belser and Castro (1971) have described the metabolism of 3-chloroallyl alcohol by a soil bacteria to CO_2. Previously, Castro and Belser (1966) have described the hydrolysis of 1,3-dichloropropene to 3-chloroallyl alcohol. Additionally, Castro and Bartnicki (1965) have described the metabolism of 3-bromopropanol by a soil bacteria. The reaction converting 3-chloroallyl alcohol to 3-chloro-1-propanol has not been described

biologically but it is chemically possible. Likewise, the conversion of 3-chloroacrylic acid to 3-chloropropionic acid and 3-hydroxypropionic acid is chemically possible but not biologically described (the 3-hydroxypropionic acid could be formed by an addition across an α-β unsaturated acid). The conversion of formylacetic acid to malonic acid has not been biologically described but it also is chemically possible. It would be expected that 3-chloro-1-propanol would follow a similar metabolism to 3-bromopropanol and that 3-chloropropionic acid and 3-hydroxypropionic acid would result. Conceivably, malonic acid and eventually CO_2 would be in this degradation scheme. Potentially, one or several of these compounds could be present in fumigated soil for the plant to absorb. Hence, 3-chloroallyl alcohol, 3-chloropropionic acid, 3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid were used along with Telone, to ascertain their effects on the uptake of 3-^{14}C-HMG into carotenoids and the degradation of carotenoids by lipoxidase.

Telone, cis-1,3-dichloropropene and trans-1,3-dichloropropene can form free radical chlorides which would be expected to be toxic to the plant as well as enzymes. The cis isomer appears to be more toxic than the trans isomer of 1,3-dichloropropene (Table 8, Figure 6) as was found by Moje (1959). The toxicity may relate to the ability of the organochloride to undergo S_N2 type reactions. In addition, Kennedy, Buckman and Wood (1969) have shown that carbens are formed in the dehalogenation of some organochloride complexes. Telone has also been shown to be phytotoxic by

itself and the corresponding 3-chloroallyl alcohol has biocide activity (Belser and Castro, 1971) which exhibits phytotoxicity at very low levels. If the organohalide survives the nucleophillic sites in the cell wall, it can proceed to react with iron to oxidize it to the Fe^{+3} state and interrupt the respiratory chain (Castro, 1966). Cobalt has also been oxidized by organohalides as suggested by Wood, Kennedy and Wolfe (1968). The above possibilities are all plausible explanations for the inhibition of 3-^{14}C-HMG uptake by excised etiolated maize shoots. Soluble extracts were not tried because of the results at the shoot level. The reason being is that if the above toxic mechanisms are valid, they would be even more toxic to the cell's organelles.

The inhibition of carotenoid biosynthesis by 3-chloro-1-propanol 3-chloropropionic acid and 3-hydroxypropionic acid may relate to the increased uptake of these compounds by the plant. They are more readily soluble in water and as suggested by Litterst, Lichtenstein and Kajiwara (1969), they become more effective inhibitors because more material reaches the target (mass action principle). The apparent activation of 3-^{14}C-HMG incorporation into carotenoids by 2×10^{-7} M 3-chloroallyl alcohol is not readily explainable. The effects of malonic acid on HMG uptake were not expected but the resulting inhibition of respiration may have interfered with energy synthesis and thus decreased the amount of energy available to synthesize carotenoids (malonic acid is a classical competitive inhibitor of succinic dehydrogenase in the TCA cycle).

The effects of Telone, 3-chloroallyl alcohol and malonic acid on the uptake of 2-^{14}C-MVA into carotenoids can be explained in a similar fashion as with 3-^{14}C-HMG. Telone and 3-chloroallyl alcohol are phytotoxic and have the ability to form free radicals (chloride and carben) and malonic acid is an inhibitor of respiration.

The effects of Telone, cis-1,3-dichloropropene and trans-1,3-cichloropropene on lipoxidase activity are very inhibitory. Free radical chloride ions are highly reactive and could attack some of the amino acids in a protein (imidazole ring of Histidine). Carbenes could add to any double bond system or to ring systems such as the imidazole ring or amino groups. Both free radicals could induce conformational changes in the protein to decrease enzyme activity. Lipoxidase was very sensitive to 3-chloroallyl alcohol as can be seen by the low concentrations used to inhibit the enzyme. The apparent toxicity of this compound may relate to free radical formation or its ability to induce molecular rearrangements. 3-Chloropropionic acid exhibited inhibitory properites toward lipoxidase at two of the four concentrations. The apparent activation at the 3×10^{-8} M concentration is not readily explained. The inhibition of lipoxidase by 3-hydroxypropionic acid and 3-chloro-1-propanol at most levels again is probably due to toxicity of the compounds to the enzyme protein and not the binding of the compounds to the active site of the enzyme. (Telone, cis and trans-1,3-dichloropropene, 3-chloroallyl alcohol, 3-chloropropionic acid, 3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid are not sterically similar to the cis,cis-1,4-pentadiene system.) The inhibition of

lipoxidase by malonic acid has not been previously reported. Since repeated experiments gave similar results, malonic acid must affect lipoxidase in some unknown way.

The apparent increase in total plant carotenoid in plants grown on fumigated soils does not appear to be solely the effect of Telone or one of the related compounds on the biosynthesis or degradation of the carotenoids. Since both processes, biosynthesis and degradation of carotenoids, are inhibited, the supposition that Telone or one of its related compounds is exerting a direct effect on the plant becomes less plausible. However, the biosynthesis of carotenoids is inhibited at higher concentrations of Telone and related compounds than the degradation process. The inhibitory concentrations of cis-1, 3-dichloropropene and 3-chloroallyl alcohol are one-hundred fold less for inhibition of degradation than for inhibition of biosynthesis (1.5×10^{-9} vs 2×10^{-7} M). The difference between these two treatment levels (1.5×10^{-9} vs 2×10^{-7} M) may result in a rate differential favoring the long term increase in carotenoids in the plant due to a greater suppression of lipoxidase activity at exposed concentrations within the plant. Alternatively, Telone or one of its related compounds may be absorbed by the plant and further metabolized to some as of yet unknown compound. The ramification of the unknown compound could be expressed as a resulting increase in carotenoid content of the plant.

Table 1. Absorption maxima of carotenoids

Carotene	Solvent	Absorption maxima (nm)		
α^*	Acetone	424	448	476
R_f 0.290	Acetone	422	444	472
β^*	Acetone	427	454	480
R_f 0.916	Acetone	426	452	478

* Standard α and β-carotene.
R_f 0.290 α-carotene from extract.
R_f 0.916 β-carotene from extract.

Source: Hiyama, Nishimura , and Chance, 1969.

Table 2. Incorporation of 3-^{14}C-HMG and 2-^{14}C-MVA into carotenes

	Excised etiolated seedlings	Crude extract	Soluble extract	Excised etiolated seedlings	Soluble extract
Radioactive substrate	3-^{14}C-HMG	3-^{14}C-HMG	3-^{14}C-HMG	2-^{14}C-MVA	2-^{14}C-MVA
Total activity added (dpm)	1.16×10^5	1.16×10^5	1.44×10^5	2.4×10^5	2.4×10^5
Incubation period (hrs)	48 hr	24 hr	24 hr	48 hr	24 hr
Total activity in nonsaponifiable fraction (dpm)	7945[a]	11,237	15,242	161,018	144,188
Incorporation into β-carotene (dpm)	2249	2213	1782	8,648	----
% incorporation of label into total nonsaponifiable fraction	6.84	9.68	13.13	67.09	60.07
% incorporation into total β-carotene fraction	28.3	19.7	11.7	5.4*	---

*5.4% unusually low except that there were a large number of other carotenoids produced by maize upon MVA feeding.
[a]dpm/2 gm tissue.

Table 3. Comparison of incorporation of 3-^{14}C-HMG and 2-^{14}C-MVA into carotenoids

	Substrate		
	HMG (dpm)	MVA (dpm)	% HMG of MVA
Total activity added	1.16×10^5	2.4×10^5	
Total activity in nonsaponifiable ether soluble fraction at time			
0 hr	——	——	——
24 hr	33,110[a]	77,886*	87.9
36 hr	36,993	161,018	47.9
48 hr	41,972	147,172	58.9
66 hr	41,793	153,480	56.3
Final incorporation into nonsaponifiable fraction in %	36.0	63.9	——

*MVA was divided by 2.068 to give comparable initial inoculation level equivalent to HMG.

[a]dpm/7 gm tissue.

Table 4. Effect of additional MVA on the rate of incorporation of 3-^{14}C-HMG into carotenes

	Excised etiolated seedlings		Soluble extract	
Radioactive substrate	3-^{14}C-HMG	3-^{14}C-HMG	3-^{14}C-HMG	3-^{14}C-HMG
Cold substrate added	----	MVA	----	MVA
Total activity added (dpm)	1.16×10^5	1.16×10^5	1.44×10^5	1.44×10^5
Incubation period (hr)	48	48	24	24
Total activity in nonsaponifiable fraction (dpm)	7945	4854	15,242	2611

Table 5. Incorporation of 3-^{14}C-HMG into carotenoids of maize as influenced by reducing agents in the incubation mixture alone and in extraction medium and incubation mixture

	Incubation		Extraction and incubation	
	Crude extract	Soluble extract	Crude extract	Soluble extract
	(radioactivity, cpm)			
- GSH	2312	1104	1156	811
+ GSH	7374	7903	12,032	11,799
- 2-mercaptoethanol	1712	1075	927	2645
+ 2-mercaptoethanol	9655	3345	14,157	12,597

Table 6. Effects of Telone and related compounds on incorporation of 3-^{14}C-HMG into carotenoids

Treatment		
Compound	Concentration (M)	Radioactivity (dpm)
Control	----	3994 ± 191
Telone[a]	2×10^{-5}	1402 ± 230**
	2×10^{-6}	1889 ± 224**
	2×10^{-7}	2447 ± 123*
	2×10^{-8}	1495 ± 118**
3-chloroallyl alcohol	2×10^{-5}	2178 ± 271**
	2×10^{-6}	4456 ± 197^{ns}
	2×10^{-7}	2263 ± 145**
3-chloropropionic acid	2×10^{-5}	2672 ± 92*
	2×10^{-6}	3348 ± 90^{ns}
	2×10^{-7}	4220 ± 105^{ns}
	2×10^{-8}	3739 ± 98^{ns}
3-hydroxypropionic acid	2×10^{-5}	1981 ± 85**
	2×10^{-6}	1847 ± 61**
	2×10^{-7}	2352 ± 101*
	2×10^{-8}	2320 ± 68*
3-chloro-1-propanol	2×10^{-5}	2079 ± 92**
	2×10^{-8}	1918 ± 81**
Malonic acid	2×10^{-5}	2031 ± 111**

* = Significance at the .05 level.
** = Significance at the .01 level.
ns = Non-significant at the .05 level.
Mean values of four experimental units.

[a] Molarity of Telone was based on the F.W. of 1,3-dichloropropene.

Table 7. Effects of Telone and related compounds on incorporation of 2-^{14}C-MVA into carotenoids

Treatment		
Compound	Concentration (M)	Radioactivity (dpm)
Control	----	21598 ± 1200
Telone[a]	2×10^{-5}	13106 ± 1470**
3-chloroallyl alcohol	2×10^{-5}	17474 ± 1380*
Malonic acid	2×10^{-5}	16084 ±1656*

* = Significance at the .05 level.
** = Significance at the .01 level.
Mean values of four experimental units.
[a]Molarity of Telone was based on the F.W. of 1,3-dichloropropene.

Table 8. Effect of Telone and related compounds on lipoxidase activity

Treatment		Specific Activity
Compound	Concentration (M)	(△ O. D. /Min/ 10 mg protein)
Control	----	.057
Telone[a]	3×10^{-8}	.029**
	6×10^{-8}	.027**
	1.2×10^{-7}	.018**
cis-1,3-dichloropropene	3×10^{-8}	.024**
	6×10^{-8}	.016**
	1.2×10^{-7}	.010**
trans-1,3-dichloropropene	3×10^{-8}	.046[ns]
	1.5×10^{-7}	.040*
	3×10^{-7}	.032*
3-chloroallyl alcohol	3×10^{-10}	.068[ns]
	7.5×10^{-10}	.050[ns]
	1.5×10^{-9}	.032*
	3×10^{-8}	.022**
3-chloropropionic acid	3×10^{-8}	.073*
	6×10^{-8}	.044*
	1.2×10^{-7}	.067[ns]
	2.4×10^{-7}	.029**
3-hydroxypropionic acid	3×10^{-8}	.042*
	1.5×10^{-7}	.039*
	3×10^{-7}	.029**
	6×10^{-7}	.025**

Table 8. Continued

Treatment		Specific Activity
Compound	Concentration (M)	(△O.D./Min/ 10 mg protein)
3-chloro-1-propanol	3×10^{-8}	.060 ns
	1.5×10^{-7}	.037*
	3×10^{-7}	.018**
	6×10^{-7}	.008**
Malonic acid	3×10^{-8}	.035*
	1.5×10^{-7}	.026**
	3×10^{-7}	.017**

* = Significance at the .05 level.

** = Significance at the .01 level.

ns = Non-significance at the .05 level.

Mean values of eight replications.

[a]Molarity of Telone was based on the F.W. of 1,3-dichloropropene.

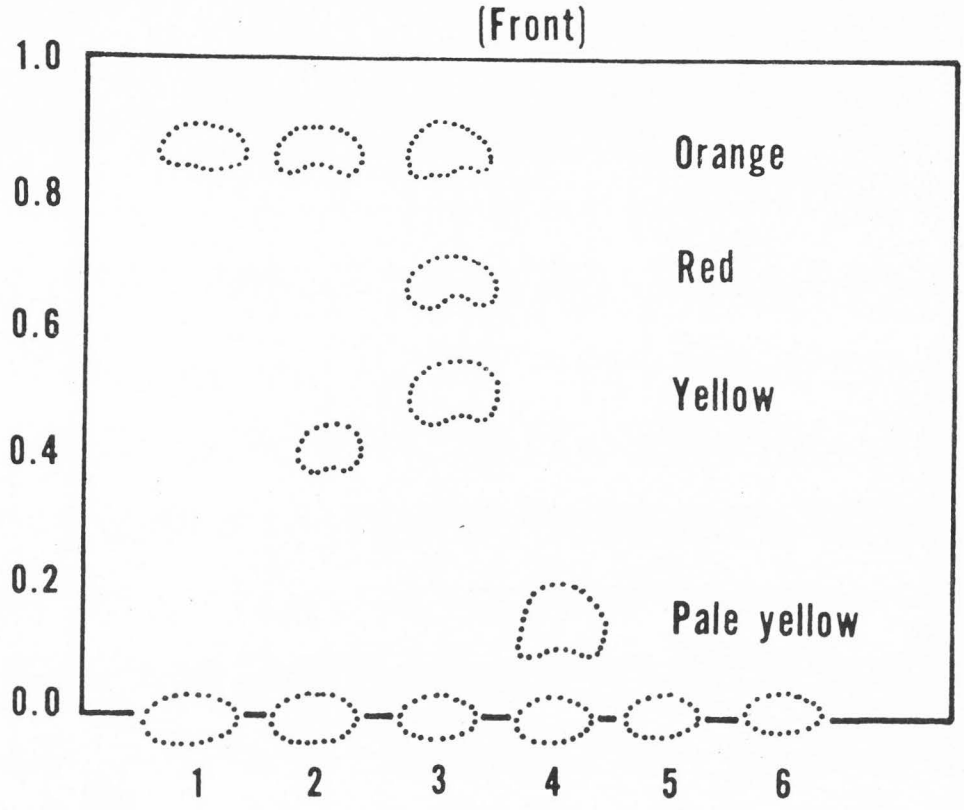

Figure 1. Radioautogram of reaction products from TLC.

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1 = β-carotene standard
2 = Tissue + HMG
3 = Tissue + MVA
4 = Crude extract + HMG
5 = HMG
6 = MVA

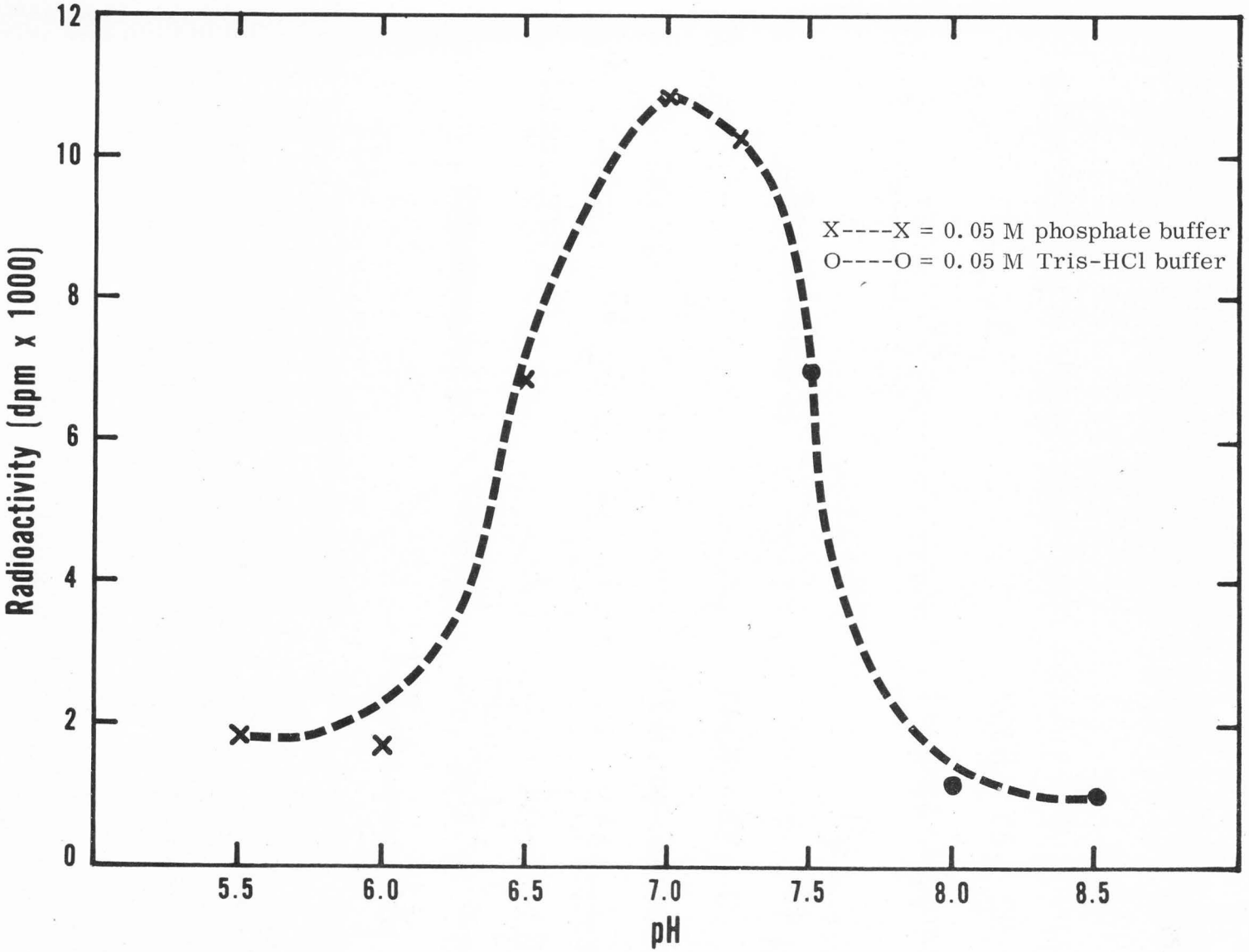

Figure 2. Effect of pH on incorporation of 3-^{14}C-HMG into carotenoids.

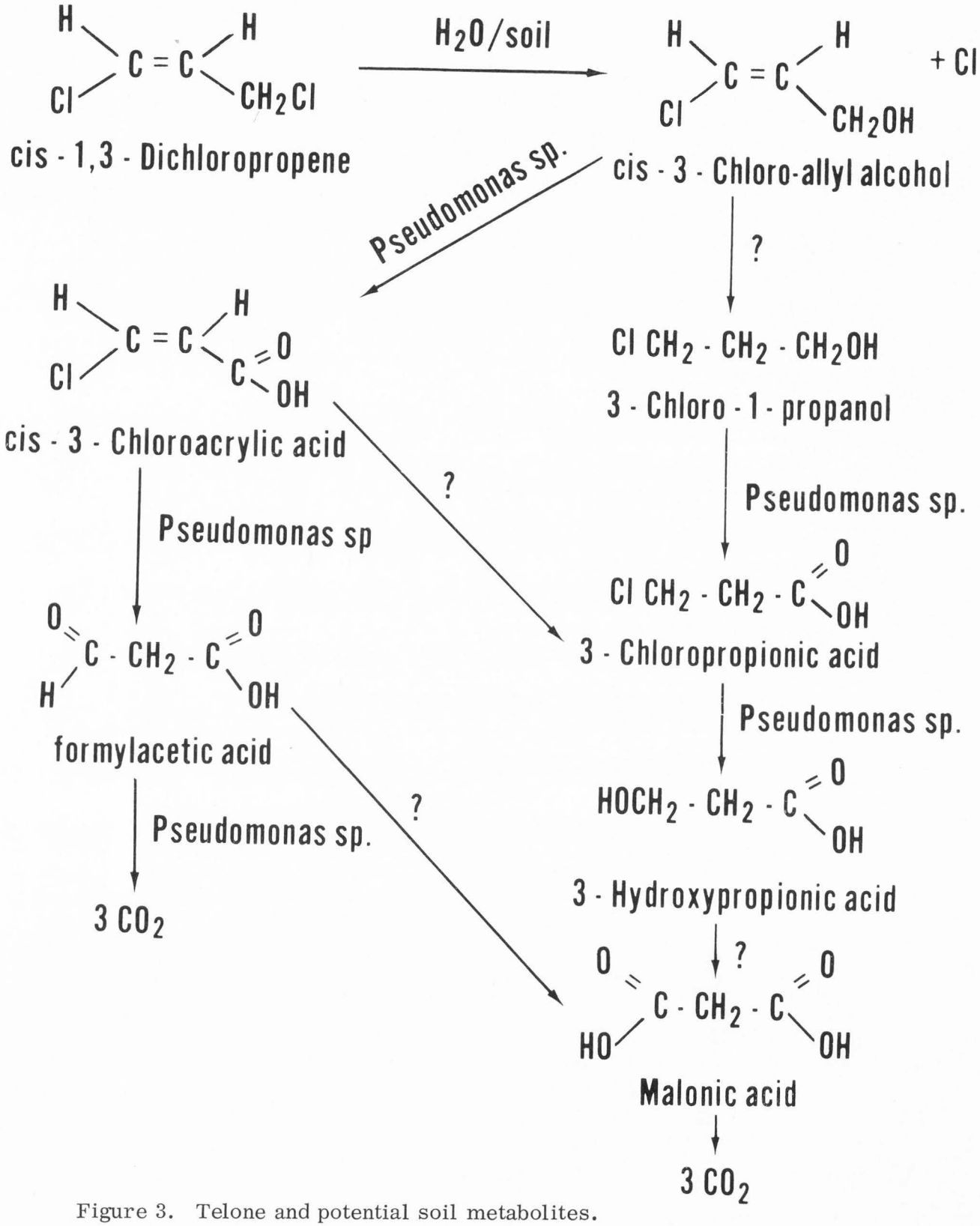

Figure 3. Telone and potential soil metabolites.

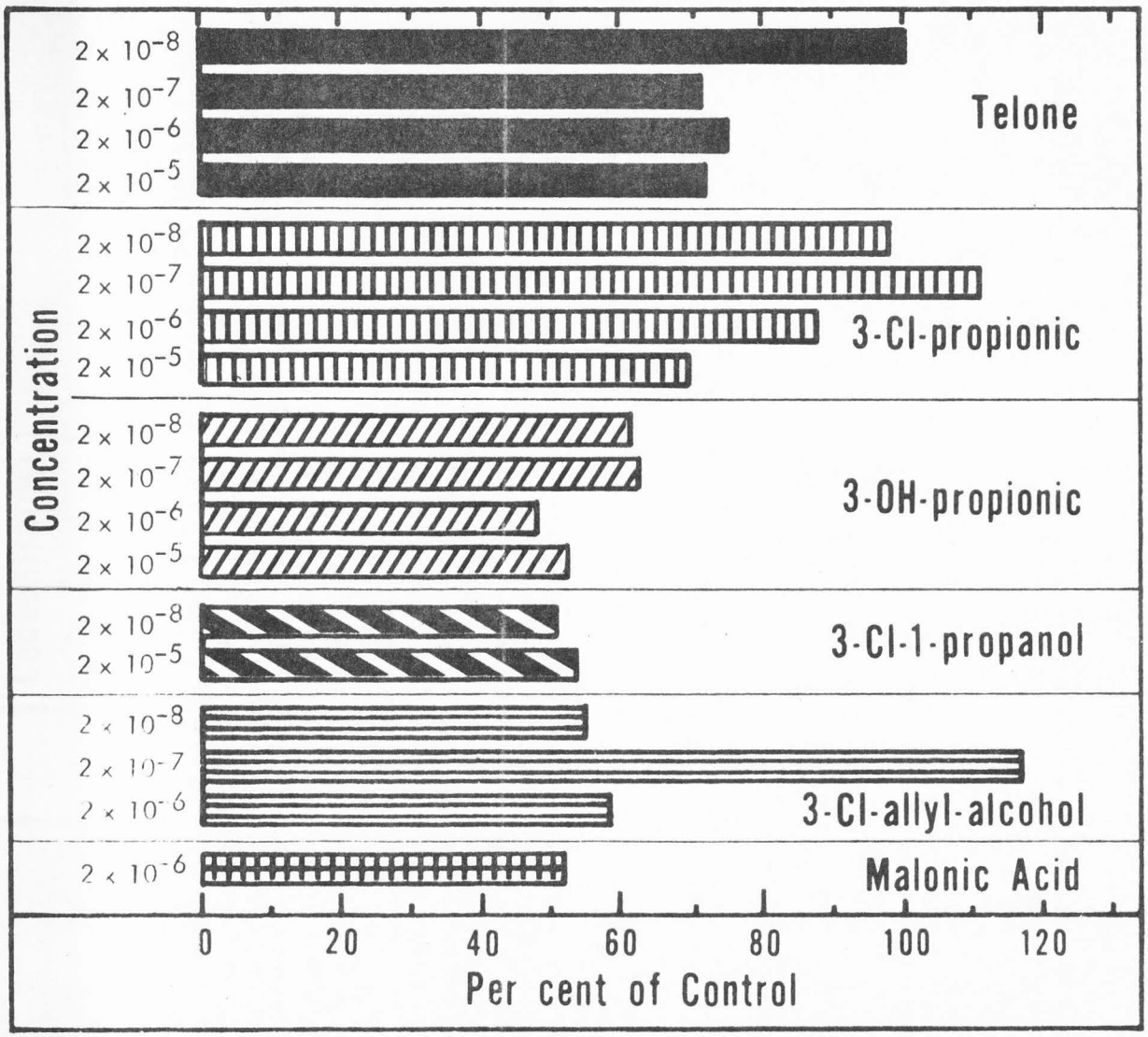

Figure 4. Effect of Telone and related compounds on incorporation of 3-^{14}C-HMG into carotenoids in maize shoots.

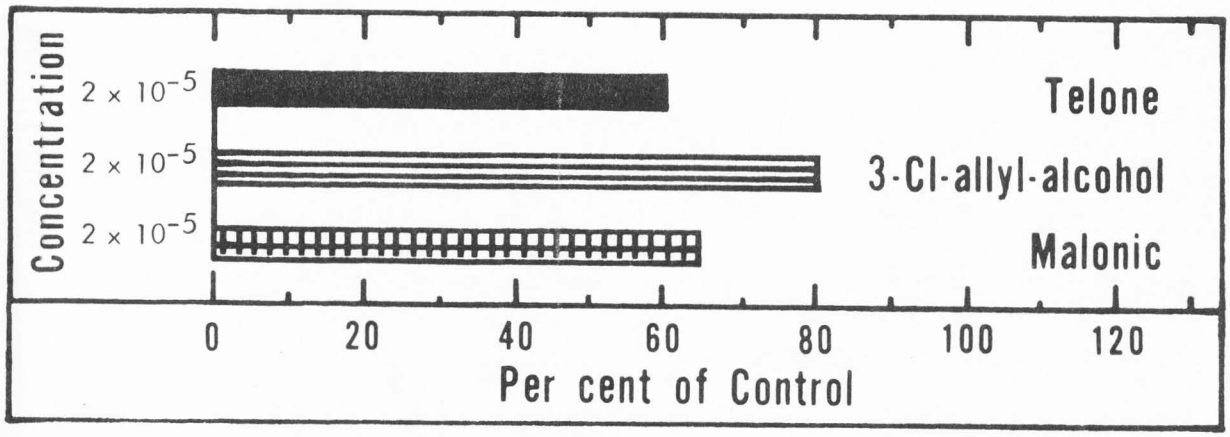

Figure 5. Effect of Telone and related compounds on incorporation of 2-^{14}C-MVA into carotenoids in maize shoots.

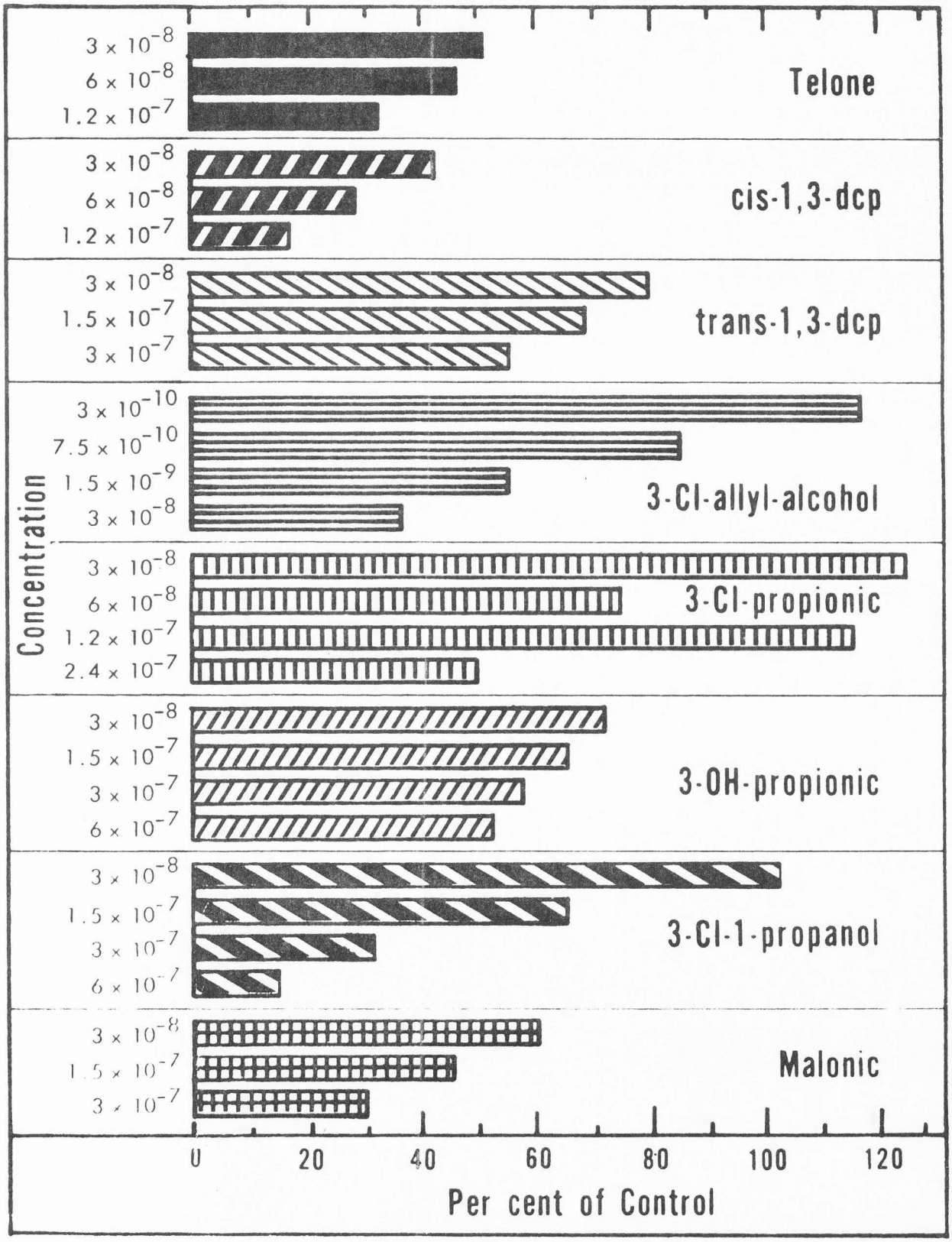

Figure 6. Effect of Telone and related compounds on lipoxidase activity.

SUMMARY AND CONCLUSIONS

The effects of Telone (a mixture of cis-1,3-dichloropropene, trans-1,3-dichloropropene and other halogenated hydrocarbons) on the biosynthesis and degradation of carotenoids in plants was investigated. The biosynthetic studies were based on the incorporation of 3-^{14}C-β-hydroxy-β-methylglutaric acid (3-^{14}C-HMG) into carotenoids of maize. The degradation studies employed a crude soluble extract of tomato lipoxidase to measure the affects of Telone on the decomposition of unsaturated fatty acids.

The incorporation of 3-^{14}C-HMG into carotenoids of excised etiolated maize shoots, crude maize extracts and soluble maize extracts was investigated. The role of HMG in carotenoid biosynthesis in plants had not been heretofore investigated. HMG and HMG-CoA were incorporated into carotenoids directly and not degraded to acetate or acetyl-CoA first and then back up into isoprenoids. Results indicated that HMG-CoA reductase and HMG activating enzyme were present in maize. The soluble maize extract showed an optimum pH of 7.0 for HMG incorporation. Endogenous metabolites such as mevalonic acid (MVA) in the reaction mixture decreased the rate of incorporation of HMG into carotenoids. HMG-CoA reductase may be a site of regulation in carotenoid biosynthesis in the plant.

The effects of Telone, 3-chloroallyl alcohol, 3-chloropropionic acid, 3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid on HMG

incorporation into carotenoids of excised etiolated maize shoots were studied. All of the compounds tried, significantly decreased the rate of incorporation of 3-^{14}C-HMG into carotenoids.

The effects of Telone, cis-1,3-dichloropropene, trans-1,3-hydroxypropionic acid, 3-chloro-1-propanol and malonic acid on the activity of tomato lipoxidase were studied. All compounds tested including malonic acid were significantly inhibitory to the activity of the enzyme. The compounds cis-1, 3-dichloropropene and 3-chloroallyl alcohol were especially effective inhibitors.

The apparent increase in carotenoid level of plants grown on Telone fumigated soils does not appear to be the result of a direct effect of Telone on the plant. Both the biosynthesis and degradation of carotenoids are inhibited by Telone and related compounds but not at the same treatment levels. The differential between the inhibition of the biosynthetic rate as opposed to the inhibition of the degradative rate (degradative being inhibited at lower treatment levels) could account for an eventual increase in the total carotenoid. Alternatively, there are two additional distinct possibilities for the increased carotenoid content in the plant. First, Telone is metabolized by soil microorganisms at the beginning of the growing season of the plant and by the time the life cycle of the microorganism is completed, large pools of organic metabolites are released near the plant's root system which may be absorbed and utilized in the synthesis of carotenoids. Second, the plant may be

absorbing and metabolizing Telone and the results of that metabolism may be expressed by the increased levels of carotenoids in the plant.

LITERATURE CITED

Altman, J., and S. Lawlor. 1966. The effects of some chlorinated hydrocarbons on certain soil bacteria. Journal of Applied Bacteriology 29:260-265.

Altman, J., and K. M. Tsue. 1965. Changes in plant growth with chemicals used as soil fumigants. Plant Disease Reporter 49:600-602.

Archer, B. L., B. G. Audley, E. G. Cockbain, and G. P. McSweeney. 1963. The biosynthesis of rubber: Incorporation of mevalonate and isopentenyl pyrophosphate into rubber by Heva brasiliensis latex fractions. Biochemical Journal 89:565-574.

Arnon, D. I., and D. R. Hoagland. 1940. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Science 50:463-483.

Bartnicki, E. W., and C. E. Castro. 1969. Biodehalogenation. The pathway for transhalogenation and stereochemistry of epoxide formation for halohydrins. Biochemistry 8:4677-4680.

Belser, N. O., and C. E. Castro. 1971. Biodehalogenation. The metabolism of the nematocides cis and trans-3-chloroallyl alcohol by a bacterium isolated from soil. Journal of Agricultural and Food Chemistry 19:23-26.

Brodie, J., and J. W. Porter. 1960. The synthesis of mevalonic acid by non-particulate avian and mammaliam enzyme systems. Biochemical and Biophysical Research Communications 3:173-177.

Brodie, J., G. Wasson, and J. W. Porter. 1963. The participation of malonyl coenzyme A in the biosynthesis of mevalonic acid. Journal of Biological Chemistry 238:1294-1301.

Brodie, J., G. A. Wasson, and J. W. Porter. 1964. Enzyme-bound intermediates in the biosynthesis of mevalonic and palmitic acids. Journal of Biological Chemistry 239:1346-1356.

Bucher, N. L., P. Overath, and F. Lynen. 1960. β-hydroxy-β-methylglutaryl coenzyme A reductase. Cleavage and condensing enzymes in relation to cholesterol formation in rat liver. Biochemica et Biophysica Acta 40:491-501.

Burch, R. E., H. Rudney, and J. J. Irias. 1964. The activation of and metabolism of β-hydroxy-β-methylglutaric acid. Journal of Biological Chemistry 239:4111-4116.

Caseley, J. C., and F. E. Broadbent. 1968. The effect of five fungicides on soil respiration and some nitrogen transformations in Yolo fine sandy loam. Bulletin of Environmental Contamination and Toxicology 3:58-64.

Casida, J. E., and L. Lykken. 1969. Metabolism of organic pesticide chemicals in higher plants. Annual Review of Plant Physiology 20:607-636.

Castro, C. E. 1966. The rapid oxidation of iron (II) porphyrins by alkyl halides. A possible mode of intoxication of organisms by alkyl halides. Journal of the American Chemical Society 86:2310-2311.

Castro, C. E., and E. W. Bartnicki. 1965. Biological cleavage of carbon-halogen bonds. Metabolism of 3-bromopropanol by Pseudomonas sp. Biochemical et Biophysica Acta 100:384-392.

Castro, C. E., and E. W. Bartnicki. 1968. Biodehalogenation. Epoxidation of halohydrins, epoxide opening and transhalogenation by a Flavobacterium sp. Biochemistry 7:3213-3218.

Castro, C. E., and N. O. Belser. 1966. Hydrolysis of cis and trans-1,3-dichloropropene in wet soil. Journal of Agricultural and Food Chemistry 14:69-70.

Castro, C. E., and N. O. Belser. 1968. Biodehalogenation. Reductive dehalogenation of the biocides ethylene dibromide, 1,3-dibromo-3-chloropropane and 2,3-dibromobutane in soil. Environmental Science and Technology 2:779-783.

Chesterton, C. J., and R. G. O. Kekwick. 1968. Formation of Δ^3-isopentenyl monophosphate and pyrophosphate in latex of Hevea brasiliensis. Archiv Biochemica et Biophysica 125:76-85.

Cole, H., D. MacKenzie, C. B. Smith, and E. L. Bergman. 1968. Influence of various persistant chlorinated insecticides on the macro and micro element constituents of Zea mays and Phaseolus vulgaris growing in soil containing various amounts of these chemical. Bulletin of Environmental Contamination and Toxicology 3:141-154.

Cooper, G. Z., and C. R. Benedicts. 1967. Compartmentalization of acetate pools in carotene synthesis. Plant Physiology 42(suppl.):s44.

Cotner, R. C., R. H. Hamilton, R. O. Mumma, and D. E. Prear. 1968. Halogenated pesticides. Localization of dieldrin in wheat tissue. Journal of Agricultural and Food Chemistry 16:608-610.

Durr, I. F., and H. Rudney. 1960. The reduction of β-hydroxy-β-methylglutaryl coenzyme A to mevalonic acid. Journal of Biological Chemistry 235:2573-2578.

English, H., and J. E. Devay. 1964. Influence of soil fumigation on growth and canker resistance of young fruit trees in California. Down to Earth 20:6-8.

English, H., J. E. Devay, O. Lilleland, and J. R. Davis. 1961. Effects of certain soil treatments on development of bacterial canker in peach trees. Phytopathology 51:65.

Ferguson, J. J., I. F. Durr, and H. Rudney. 1958. Enzymatic reduction of β-hydroxy-β-methylglutaryl CoA (HMG-CoA) to mevalonic acid. Federation Proceedings 17:219.

Fletcher, F. W. 1956. Telone. The new Dow soil fumigant containing dichloropropene. Down to Earth 11:6-7.

Gabelman, W. H. 1970. Alleviating the effects of pollution by modifying the plant. HortScience 5:16-18.

Goldman, P., G. W. A. Milue, and D. B. Keister. 1968. Carbon-halogen bond cleavage. Journal of Biological Chemistry 243:428-434.

Goodwin, T. W. 1964. Modern methods in plant analysis. Edited by K. Paech and M. V. Tracey. Vol. III. Enzymes of vitamin metabolism. Springer-Verlag, Berlin.

Goodwin, T. W. 1969. Perspectives in phytochemistry. Edited by J. B. Harborne and T. Swain. Recent investigations in the biosynthesis of carotenoids and terpenes. Academic Press, London.

Grossman, S., A. BenAziz, P. Budowiski, I. Ascarelli, A. Gertler, Y. Birk, and A. Bondi. 1969. Enzymatic oxidation of carotene and linoleate by alfalfa. Extraction and separation of active fractions. Phytochemistry 8:2287-2293.

Harris, C. R., and W. W. Sans. 1967. Adsorption of organochlorine insecticide residues from agricultural soils by root crops. Journal of Agricultural and Food Chemistry 15:861-863.

Hepper, C. M., and B. G. Audley. 1969. The biosynthesis of rubber from β-hydroxy-β-methylglutaryl coenzyme A in Heva brasiliensis latex. Biochemical Journal 114:379-386.

Hill, H. M. S., S. P. S. Shah, and L. J. Rogers. 1970. Incorporation of 2-^{14}C-glyoxylate, 2-^{14}C-acetate and 2-^{14}C-mevalonic acid into terpenoids during ripening of tomato fruits. Phytochemistry 9:749-753.

Hilz, H., J. Knappe, E. Ringlemann, and F. Lynen. 1958. Methyl glutaconase, eine neue hydratase, die am stoffwechsel verweighter carbonsauren beteiligt ist. Biochemische Zeitschrift 329:476-489.

Hiyama, T., M. Nishimura, and B. Chance. 1969. Determination of carotenes by thin-layer chromatography. Analytical Biochemistry 29:339-350.

Jurinak, J. J. 1957. Adsorption of 1,2-dibromo-3-chloropropane vapor by soils. Journal of Agricultural and Food Chemistry 5:598-601.

Jurinak, J. J., A. L. Brown, and P. E. Martin. 1960. Extraction and determination of ethylene dibromide in soils. Journal of Agricultural and Food Chemistry 8:113-115.

Kawachi, T., and H. Rudney. 1970. Solubilization and purification of β-hydroxy-β-methylglutaryl coenzyme A reductase from rat liver. Biochemistry 9:1700-1705.

Kearney, P. C., D. D. Kaufman, and M. L. Ball. 1964. The enzyme for dehalogenation of 2,2-dichloropropionate. Biochemical and Biophysical Research Communications 14:29-33.

Kennedy, F. S., T. Buckman, and J. M. Wood. 1969. Carbenoid intermediates from the photolysis of haloalkylcobalamins. Biochemica et Biophysica Acta 177:661-663.

Kies, M. W., J. L. Haining, E. P. Pistorius, D. H. Schroeder, and B. Axelrod. 1969. On the question of the identity of soybean lipoxidase and carotene oxidase. Biochemical and Biophysical Research Communications 36:312-315.

Kirtley, M. E., and H. Rudney. 1967. Some properties and mechanism of action of the β-hydroxy-β-methylglutaryl coenzyme A reductase of yeast. Biochemistry 6:230-238.

Knappe, J., E. Ringlemann, and F. Lynen. 1959. Ube die β-hydroxy-β-methylglutaryl reduktase der hefe. IX. Zer biosynthese der terpene. Biochem Zeitschrift 332:195-213.

Knauss, H. J., H. W. Porter, and G. Wasson. 1959. The biosynthesis of mevalonic acid from 1-^{14}C-acetate by rat liver enzyme system. Journal of Biological Chemistry 234:2835-2840.

Leistra, M. 1970. Distribution of 1,3-dichloropropene over phases in soil. Journal of Agricultural and Food Chemistry 18:1124-1126.

Lichtenstein, E. P. 1960. Insecticidal residues in various crops grown in soil treated with abnormal rates of aldrin and heptachlor. Journal of Agricultural and Food Chemistry 8:448-451.

Lichtenstein, E. P., and J. R. Corbett. 1969. Enzymatic conversion of aldrin to dieldrin with subcellular components of pea plants. Journal of Agricultural and Food Chemistry 17:589-594.

Lichtenstein, E. P., T. W. Fuhremann, N. E. A. Scopa, and R. F. Shrentny. 1967. Translocation of insecticides from soils into pea plants. Effect of detergent LAS on translocation and plant growth. Journal of Agricultural and Food Chemistry 15:864-869.

Lichtenstein, E. P. W. F. Millington, and G. T. Crowley. 1962. Effects of various insecticides on growth and respiration of plants. Journal of Agricultural and Food Chemistry 10:251-256.

Lichtenstein, E. P., and G. R. Myrdal. 1965. Adsorption of insecticidal residues from contaminated soils into five carrot varieties. Journal of Agricultural and Food Chemistry 13:126-131.

Lichtenstein, E. P., and K. R. Schulz. 1959. Persistance of some chlorinated hydrocarbon insecticides as influenced by soil types, rate of application and temperature. Journal of Economic Entomology 52:125-131.

Lichtenstein, E. P., and K. R. Schulz. 1960. Translocation of some chlorinated hydrocarbon insecticides into the aerial parts of pea plants. Journal of Agricultural and Food Chemistry 8:452-456.

Lichtenstein, E. P., and K. R. Schulz. 1965. Residues of aldrin and heptachlor in soils and their translocation into various crops. Journal of Agricultural and Food Chemistry 13:57-63.

Lichtenstein, E. P., K. R. Schulz, T. W. Fuhremann, and 1970. Degradation of aldrin and heptachlor in field soils during a ten-year period. Translocation into crops. Journal of Agricultural and Food Chemistry 18:100-106.

Linn, T. C. 1967a. The demonstration and solubilization of β-hydroxy-β-methylglutaryl coenzyme A reductase from rat liver microsomes. Journal of Biological Chemistry 242:984-989.

Linn, T. C. 1967b. The effect of cholesterol feeding and fasting upon β-hydroxy-β-methylglutaryl coenzyme A reductase. Journal of Biological Chemistry 242:990-993.

Litterst, C. L., E. P. Lichtenstein, and K. Kajiwara. 1969. Effects of insecticides on growth of hela cells. Journal of Agricultural and Food Chemistry 17:1199-1203.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry 193:265-275.

Lynen, F. 1967. Biosynthetic pathways from acetate to natural products. Pure and Applied Chemistry 14:137-167.

MacKenzie, D., H. Cole, C. B. Smith, and E. L. Bergman. 1968. Influences of various soil incorporated fungicides and nematocides on macro and micro element constituents of Zea mays and Phaseolus vulgaris. Bulletin of Environmental Contamination and Toxicology 3:116-126.

Maier-Bodie, H. 1967. The aldrin and dieldrin contents of German edible carrots. Bulletin of Environmental Contamination and Toxicology 2:10-11.

Martin, J. P., and P. E. Pratt. 1958. Fumigants, fungicides and the soil. Journal of Agricultural and Food Chemistry 6:343-348.

McCants, C. B., O. E. Skogley, and G. W. Woltz. 1959. Influence of certain soil fumigation treatments on the response of tobacco to ammonium and nitrate forms of nitrogen. Proceedings of the American Society of Soil Science 23:466-469.

Moje, W. 1959. Structure and nematocidal activity of allylic and acetylenic halides. Journal of Agricultural and Food Chemistry 7:703-707.

Moje, W., J. P. Martin, and R. C. Baines. 1957. Structural effect of some organic compounds on soil organisms and citrus seedlings grown in an old citrus soil. Journal of Agricultural and Food Chemistry 5:32-36.

Neumeyer, J., D. Gibbons, and H. Trask. 1969. Pesticides. Chemical Week pp. 38-68. April 12.

Oloffs, P. C., and E. P. Lichtenstein. 1969. Epoxidation of aldrin by excised pieces of plant tissue. Journal of Agricultural and Food Chemistry 17:143-147.

Porter, J. W., and D. G. Anderson. 1967. Biosynthesis of carotenes. Annual Review of Plant Physiology 18:197-228.

Potty, V. H. 1969. Occurrence and properties of enzymes associated with mevalonic acid synthesis in the orange. Journal of Food Science 34:231-234.

Rudney, H., P. R. Stewart, P. W. Majerus, and P. R. Vagelus. 1966. The biosynthesis of β-hydroxy-β-methylglutaryl coenzyme A in yeast. V. Role of acyl carrier protein. Journal of Biological Chemistry 241: 1226-1228.

Saha, J. G., C. H. Craig, and W. K. Junzen. 1968. Organochlorine insecticide residues in agricultural soil and legume crops in Northeastern Saskatchewan. Journal of Agricultural and Food Chemistry 16:617-619.

Saha, J. G., and H. McDonald. 1967. Insecticide residues in wheat grown in soil treated with aldrin and endrin. Journal of Agricultural and Food Chemistry 15:205-207.

Salunkhe, D. K., M. Wu, M. T. Wu., and B. Singh. 1971. Effects of Telone and Nemagon on essential nutritive components and respiratory rates of carrot (Daucus carota L.) roots and sweet corn (Zea mays L.) seeds. Journal of the American Society of Horticultural Science 96:357-359.

Seely, G. R., and T. H. Meyer. 1971. The photosensitized oxidation of β-carotene. Photochemistry and Photobiology 13:27-32.

Shah, S. P. J., and L. J. Rogers. 1969. Compartmentalization of terpenoid biosynthesis in green plants. Biochemical Journal 114:395-406.

Siperstein, M., and V. M. Fagan. 1966. Feed back control of mevalonate synthesis by dietary cholesterol. Journal of Biological Chemistry 241:602-609.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco, California.

Stewart, P. R., and H. Rudney. 1966a. The biosynthesis of β-hydroxy-β-methylglutaryl coenzyme A in yeast. III. Purification and properties of the condensing enzyme thiolase system. Journal of Biological Chemistry 241:1212-1221.

Stewart, P. R., and H. Rudney. 1966b. The biosynthesis of β-hydroxy-β-methylglutaryl coenzyme A in yeast. IV. The origin of the thioester bond of β-hydroxy-β-methylglutaryl coenzyme A. Journal of Biological Chemistry 241:1222-1225.

Surrey, K. 1964. Spectrophotometric method for determination of lipoxidase activity. Plant Physiology 39:65-70.

Tams, R. K. 1945. The comparative effects of a 50-50 mixture of 1,3-dichloropropene and 1,2-dichloropropane (D-D mixture) and chloropicrin on nitrification in soil and on the growth of the pineapple plant. Soil Science 59:191-205.

Tams, R. K., and H. E. Clark. 1943. Effect of chloropicrin and other soil disinfectants on the nitrogen nutrition of the pineapple plant. Soil Science 56:245-261.

Tappel, A. L. 1962. "Lipoxidase," pp. 539-542. In S. P. Colwick and N. O. Kaplan (Eds.). Methods in enzymology, Vol. V. Academic Press, London and New York.

Theigs, B. J. 1955. Effect of soil fumigation on nitrification. Down to Earth 11:14-15.

Wensley, R. N. 1953. Microbial studies on the action of some selected soil fumigants. Canadian Journal of Botany 31:277-308.

Wheeler, W. B. 1970. Environmental factors affecting dieldrin uptake by rye. Bulletin of Environmental Contamination and Toxicology 5:463-467.

White, L. W., and H. Rudney. 1970. Biosynthesis of β-hydroxy-β-methylglutarate and mevalonate by rat liver homogenates in vitro. Biochemistry 9:2713-2724.

Whitehead, A. G., D. J. Tite, and J. E. Fraser. 1970a. The effect of D-D chloropicrin and previous crops on numbers of migrating root-parasitic nematodes and on the growth of sugar beet and barley. Annals of Applied Biology 65:351-359.

Whitehead, A. G., D. J. Tite, and J. E. Fraser. 1970b. The effect of small doses of nematocides on migratory root-parasitic nematodes and on the growth of sugar beets and barley in sandy soils. Annals in Applied Biology 65:361-375.

Williams, H. 1968. Recovery of cis and trans-dichloropropene residues from two types of soil and their detection and determination by electron capture gas chromatography. Journal of Economic Entomology 61:1432-1435.

Wolcott, A. R., F. Maciak, L. N. Shepard, and R. E. Lucas. 1960. Effects of Telone on nitrogen transformations and on growth of celery in organic soil. Down to Earth 16:10-14.

Wood, J. M., E. S. Kennedy, and R. S. Wolfe. 1968. The reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B_{12}. Biochemistry 7:1707-1713.

Wu, M., and D. K. Salunkhe. 1971. Influence of soil fumigation of Telone and Nemagon on the ultrastructure of chromoplasts in carrot roots. Experientia 27:712-713.

Wu, M., B. Singh, M. T. Wu, D. K. Salunkhe, and G. G. Dull. 1970. Effects of certain soil fumigants on essential nutritive components and the respiratory rates of carrot (Daucus carota L.) roots. HortScience 5:221-222.

Yokoyama, H., T. O. M. Nakayama, and C. O. Chichester. 1962. Biosynthesis of β-carotene by cell-free extracts of Phycomyces blakesleeanus. Journal of Biological Chemistry 237:681-686.

VITA

David Lester Berry

Candidate for the Degree of

Master of Science

Thesis: Effects of Telone and Related Compounds on the Synthesis and Degradation of Carotenoids in Plants

Major Field: Nutrition and Food Sciences

Biographical Information:

Personal Data: Born Red Bluff, California, 27 March 1944; son of Mr. and Mrs. Lester J. Berry of Davis, California.

Education: Attended primary school in Anderson, California, and Davis, California; graduated from Davis Senior High School, June, 1962; received Bachelor of Science degree from University of California at Davis in Food Science, 1967; attended graduate school University of California at Davis, 1968-1969; completed requirements for Master of Science in Food Science and Industries from Utah State University in 1971.

Professional experience: Field assistant for Dr. C. W. Schaller Dept. of Agronomy and Range Science, University of California at Davis, 1958-1964; Laboratory assistant for Dr. C. W. Schaller, 1964-1967; Wine Chemist and Tour Guide, Louis M. Martini Winery, St. Helena, California, 1965-1969; Laboratory Technician, Dept. of Food Science, University of California, Davis, California, 1967-1968; Member of American Society of Enologists, Seattle Mountaineers and Society of Sigma Xi, Utah State University Chapter; Graduate Research Assistant, Utah State University.